A Comparison of Sesquiterpene Scaffolds across Different Populations of the Tropical Marine Sponge *Acanthella cavernosa*

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Specimens of the Indo-Pacific sponge *Acanthella cavernosa* Dendy collected from locations along the Eastern coastline of Australia have been shown to contain a range of sesquiterpenes including isothiocyanate **1**, isocyanide **10**, and the isocyanates **15** and **22**. These metabolite studies have provided a basis for chemical comparisons between sponge populations from different geographic locations and between individual specimens collected from a single location.

The bright orange sponge *Acanthella cavernosa* (order Halichondrida, family Dictyonellidae), which is commonly encountered on tropical reefs in the Indo-Pacific region, including Australia, shows remarkable chemical diversity. Chemical studies on the sponge, and on related species such as *Acanthella pulcherrima* and *Acanthella klethra*, have revealed numerous metabolites characterized by the presence of nitrogen-containing functionality, typically in the form of isocyanide, isothiocyanate, isocyanate, or formamide substituents attached to a terpene skeleton. Diterpenes of the kalihinane series of metabolites or sesquiterpenes with a diverse range of skeletons have both been identified.[1,2]

Great Barrier Reef populations of *A. cavernosa* that we have studied have generally been characterized by the presence of sesquiterpene compounds alone.[3,4] Diterpene (i.e., kalihinane-derived) metabolites have been encountered only once, in a collection of sponges from the Wistari channel at Heron Island.[3] In contrast, *A. cavernosa* from Indo-Pacific locations such as Japan, Guam, the Philippines, Thailand, or Fiji more commonly contains diterpene metabolites, although sesquiterpene metabolites are also found.[1,2] It may be that there are taxonomic differences between the sesquiterpene- and diterpene-containing sponges that are not yet fully apparent using the current suite of depauperate morphological characters used to differentiate sibling species of *Acanthella*. Alternatively, the differing chemistry may result from a response to environmental factors such as habitat or perhaps is influenced by the microbial community that may be associated with the sponge. In preparation for biosynthetic studies on *A. cavernosa*, we evaluated the chemical diversity of this common species with respect to collection site. In this paper we explore subtle chemical differences between specimens of *A. cavernosa* collected at the same location and between samples from different collection sites. We also provide full characterization of some metabolites from this sponge species.

Results and Discussion

In our study, sponges were collected from three dive sites (Tani's Reef, Coral Gardens, both at the Gneerings Reef, and Mudjimba Island) offshore from Mooloolaba, South-East Queensland, at a depth of 10–20 m. We first investigated the chemistry of individual sponge specimens. Ten sponges were collected individually from approximately the same depth during a single dive at the Tani's Reef site. Each sponge was extracted with CH_2Cl_2/MeOH as described in the Experimental Section, and the resulting crude extracts were subjected to GC-MS and ^{1}H NMR analysis. On the basis of the similarity of the GC traces, six of the 10 samples were combined to give an extract coded as TR1 and the remaining four extracts combined to give extract TR2. These two organic extracts were then carefully purified by SiO_2 flash chromatography followed by SiO_2 HPLC to afford an array of sesquiterpene compounds whose structures were assigned on the basis of extensive spectroscopic analysis and by comparison with literature data. Sample TR1 afforded 10-isothiocyanatoguai-6-ene (**1**) along with the known axisothiocyanate-2 (**2**),[5,6] 1-isothiocyanatoaromadendrane (**3**),[7] the epimeric 10-isothiocyanato-4-cadinenes **4** and **5**, [4,8] acanthene B (**6**),[9] 7-isothiocyanato-7,8-dihydro-α-bisabolene (**7**),[10] 10α-isothiocyanatoalloaromadendrane (**8**),[11] and 10-isothiocyanato-4-amorphene (**9**).[12] Also isolated were the new isocyanide 7-isocyano-7,8-dihydro-α-bisabolene (**10**) together with the known isocyanides axisonitrile-3 (**11**),[13,14] 1-isocyanoaromadendrane (**12**),[7] and 11-isocyano-7*βH*-eudesm-5-ene (**13**).[11] The isothiocyanate fraction of sample TR2 afforded axisothiocyanate-3 (**14**),[3,6,13,14] the guai-6-ene **1**, and the epimeric cadinenes **4** and **5**, together with aromadendranes **3** and **8**. This fraction also provided the novel isocyanate metabolite axisocyanate-3 (**15**), which we reported recently.[15] The isocyanide metabolites identified in extract TR2 were axisonitrile-3 (**11**) and 1-isocyanoaromadendrane (**12**).

The ^{1}H NMR data (Table 1) for isothiocyanate **1** showed an olefinic proton as a broad singlet at δ_H 5.16, while methyl doublets at δ_H 0.96 and 0.95 coupled to a methine signal at δ_H 2.25 suggested the presence of an isopropyl group. A methyl singlet at δ_H 1.32 corresponded to a methyl adjacent to the –NCS group, while a doublet signal at δ_H 1.04 suggested a secondary methyl group. The ^{13}C NMR spectrum showed 12 protonated carbons; there were two alkene carbons at δ_C 118.9 (CH) and 149.3 (qC) and a quaternary carbon at δ_C 68.0 assigned to the carbon adjacent to the –NCS group. These data suggested a guai-6-ene structure.[16–18] gHMBC correlations showed the signal for the methyl singlet at δ_H 1.32 had cross-peaks to δ_C 54.1, 34.6, and 68.0, which could be assigned as C-1, C-9, and C-10, respectively, and confirmed the position of Me-15 at C-10. gHMBC correlations from H-11, H-, and H-13 placed the isopropyl group at C-7, adjacent to the olefinic carbons. The DQFCOSY spectrum showed connectivities from the olefinic proton (H-6) at δ_H 5.16 to the methine proton (H-5) at δ_H 2.65 and the methine proton of the isopropyl group (H-11) at δ_H 2.25. Additional DQFCOSY correlations were from H-5 to H-1, H-4, and H-6, and between H-1 and H-6, consistent with a *W* coupling. The remaining proton and carbon assignments shown in Table 1 were based on analysis of 2D data.

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Table 1. NMR Data for Isothiocyanate **1**[a]

position	δ_C	δ_H (*J* in Hz)	gHMBC[b]	NOESY
1	54.1 CH	2.09 (ddd, 12.0, 6.9, 4.0)	C-5, C-6, C-9, C-10	H-2, H-4, Me-15
2a	23.7 CH_2	1.32 (m)		H-1, H-2a
2b		1.50 (m)		H-1, H-2a, H-3b, Me-15
3a	29.1 CH_2	1.25 (m)	C-1, C-4, C-5	
3b		1.69 (dddd, 14.0, 6.9, 1.5, 1.5)		
4	38.9 CH	2.01 (m)	C-3	H-3b, Me-14
5	42.6 CH	2.65 (m)		H-1, H-4, H-8b, Me-14
6	118.9 CH	5.16 (br, s)	C-1, C-8, C-11	H-5, H-11, Me-12, Me-13, Me-14
7	149.3 qC			
8a	24.8 CH_2	1.95 (dddd, 15.6, 6.7, 1,8, 1.8)	C-6, C-7, C-9, C-10, C-11	H-8b, Me-12, Me-13
8b		2.35 (dddd, 15.6, 13.9, 2.0, 2.0)		H-8a, H-9b
9a	34.6 CH_2	1.52 (m)	C-7, C-9	H-9b
9b		1.73 (m)	C-1, C-7, C-8, C-10, C-15	H-9a
10	68.0 qC			
11	38.0 CH	2.25 (m)	C-6, C-7, C-8, C-11, C-12	Me-12, Me-13
12	21.2 CH_3	0.96 (3H, d, 6.5)	C-7, C-11, C-13	H-6, H-8a, H-11
13	21.2 CH_3	0.95 (3H, d, 7.0)	C-7, C-11, C-12	H-6, H-8a, H-11
14	15.9 CH_3	1.04 (3H, d, 7.0)	C-3, C-4, C-5	H-4, H-5, H-6
15	30.4 CH_3	1.32 (3H, s)	C-1, C-9, C-10	H-1, H-2b
NCS	[c]			

[a] Chemical shifts referenced to $CDCl_3$ (δ_H 7.25; δ_C 77.0 ppm). [b] Shown as ^1H–^{13}C; J ^1H–^{13}C = 135 Hz, long-range ^nJ ^1H–^{13}C = 8 Hz. [c] Signal not detected.

Table 2. NMR Data for Isocyanide **10**[a]

position	δ_C	δ_H (*J* in Hz)	gHMBC[b]
1ax	23.6 CH_2	1.32 (overlapping)	
1eq		1.82 (m)	
2ax/2eq	30.7 CH_2	2.00 (2H, m)	C-4, C-6, Me-14
3	133.9 qC		
4	119.8 CH	5.36 (dd, 1.5, 3.5)	C-2, C-5, C-6, Me-14
5ax	26.3 CH_2	1.92 (m)	C-4
5eq		2.12 (m)	
6	41.9 CH	1.65 (m)	C-2, C-4, Me-15
7	63.4 (t) qC		
8a	38.3 CH_2	1.65 (m)	C-9, C-10, Me-15
8b		1.50 (m)	
9a/b	22.5 CH_2	2.10 (2H, m)	C-8, C-10, C-11
10	122.9 CH	5.07 (dddd, 7.1, 7.1, 1.4, 1.4)	C-8, C-9, Me-12, Me-13
11	132.7 qC		
12	17.7 CH_3	1.61 (3H, s)	C-10, C-11, Me-13
13	25.7 CH_3	1.67 (3H, d, 1.0)	C-10, C-11, Me-12
14	23.2 CH_3	1.63 (3H, s)	C-2, C-3, C-4
15	23.5 CH_3	1.32 (3H, t, 2.0)	C-6, C-7, C-8
NC	153.7 (t) qC		

[a] Chemical shifts referenced to $CDCl_3$ (δ_H 7.25; δ_C 77.0 ppm). [b] Shown as ^1H–^{13}C; J ^1H–^{13}C = 135 Hz, long-range ^nJ ^1H–^{13}C = 8 Hz.

Tada et al. reported 10-isothiocyanatoguai-6-ene (**16**) from an unidentified Japanese sponge, but the compound was poorly characterized by NMR, and there was no proof of relative stereochemistry other than X-ray crystallography analysis of a co-occurring isocyanide.[16] The diastereomeric isothiocyanate **17** was later isolated from a Palauan specimen of *Axinyssa aplysinoides* by He et al. and the relative stereochemistry secured by a difference NOE experiment.[17] In the NOESY spectrum of **1**, there were correlations from H-1 to H-5, confirming *cis* stereochemistry at the ring junction, and from H-1 to H-4, which confirmed the configuration at C-4. Consistent with this, H-4 had a NOESY correlation to H-5, and H-6 showed a correlation to Me-14. There was a NOESY correlation from Me-15 to H-1, which first suggested that **1** was epimeric to the Tada compound at C-10. However detailed conformational analysis using PC-Model has revealed that in each of the two isomers **1** and **16** Me-15 is pseudoequatorial, and so it could be expected to show an NOE to H-1. Consequently the relative configuration at C-10 could not be determined.

The ^1H NMR of isocyanide **10** showed two olefinic protons at δ_H 5.36 (1 H, br dd, J = 3.5 Hz, 1.5 Hz) and 5.07 (1 H, br m), respectively, three olefinic methyl signals at δ_H 1.67 (3H, s), 1.63 (3H, s), and 1.61 (3H, s), and a signal at δ_H 1.32 (3H, t, J = 2.0 Hz, coupled to ^{14}N) that was assigned to the methyl group adjacent to an isocyanide group (Table 2). In the carbon spectrum, a signal at δ_C 153.7 (t, J = 4.5 Hz, coupled to ^{14}N) confirmed the presence of the –NC function, while a signal at δ_C 63.4 (t, J = 4.6 Hz) was assigned to a carbon bearing a nitrogen substituent. There were four alkene carbons at δ_C 133.9 (qC), 132.7 (qC), 122.9 (CH), and 119.8 (CH), consistent with the presence of two trisubstituted double bonds. Comparison of these data with the literature suggested this compound had a bisabolene structure.[10,19] The alkene signal at δ_H 5.07 could be assigned to H-10 since it showed correlations to two methyl groups (δ_H 1.61 and 1.67) in the DQF COSY spectrum. The other alkene proton (H-4) at δ_H 5.36 showed a correlation to the methyl at δ_H 1.63 assigned to Me-14. Some ^1H NMR signals were not well resolved either by DQF COSY or by HSQC; therefore a 1D TOCSY experiment was undertaken. When the signal at δ_H 1.82 for H-1 was irradiated, there were correlations to H-2 at δ_H 2.0, to H-6 at δ_H 1.65, and to a signal at δ_H 1.32, also assigned to H-1. The alkene carbons at δ_C 133.9 and 132.7 could be assigned to C-3 and C-11, respectively, on the basis of gHMBC correlations to their methyl groups, while C-7 at δ_C 63.4 showed a correlation from the methyl signal at δ_H 1.32 (H-15). The remaining assignments shown in Table 2 were based on detailed analysis of 2D NMR data.

Sullivan et al. reported 7-isothiocyanato-7,8-dihydro-α-bisabolene **7** from a *Halichondria* sp. and deduced the stereochemistry as (6*R*, 7*S*) by chemical correlation with (6*R*,7*E*)-α-bisabolene.[10] This

Table 3. Collection Sites and Chemistry for Different Populations of *Acanthella cavernosa*

	compounds isolated																		
location	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**20**	**21**	**22**	**23**
Tani Reef TR1 G322184 24-10-04	X	X	X	X	X	X	X	X	X	X	X	X	X	[a]					
Tani Reef TR2 G322184 24-10-04	X		X	X	X	X		X			X	X		X	X				
Coral Gardens G319567 16-1-02			X					X			X	X		X		X			
Coral Gardens 20-9-00			X					X			X	X	X	X			X	X	
Mudjimba Island 1-12-04			X					X			X	X	X	X	X	X			X

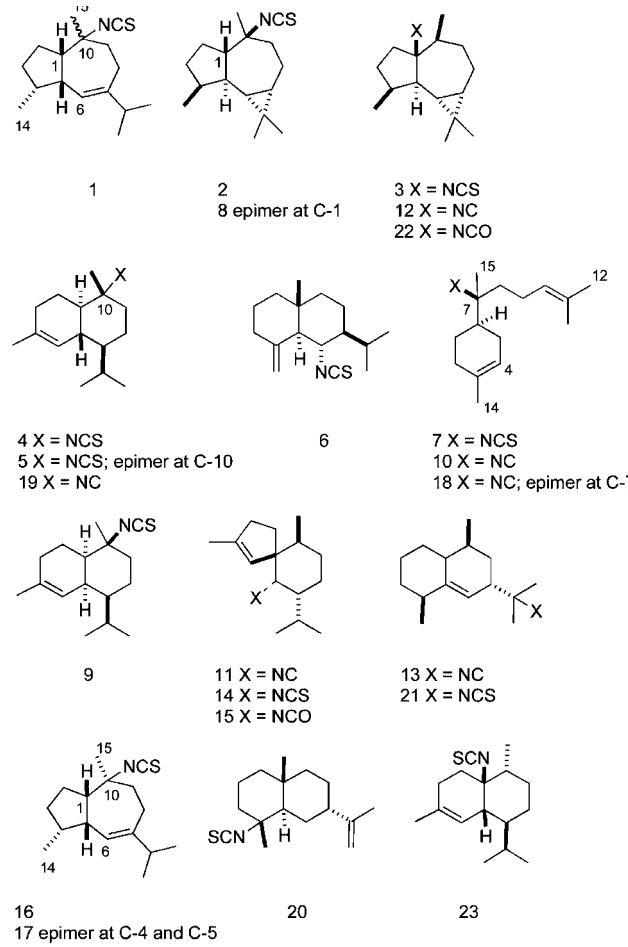
[a] Detected by GC-MS, but not isolated.

compound has also been isolated from *P. pustulosa* by Kassühlke et al.[20] Gulavita et al. isolated the bisabolene isocyanide **18** from a Hawaiian specimen of *Ciocalypta* sp.; however the stereochemistry of their compound was not rigorously proven.[19] More recently, this compound has been reported without spectroscopic data from the nudibranchs *Phyllidia pustulosa*[21] and *Phyllidia ocellata*,[22] and from *Acanthella cavernosa.*[22] The ^{1}H NMR data cited for **18** by Gulavita et al. are unreliable; when the ^{1}H NMR data for compounds **7** and **18** were compared to each other or to other bisabolene compounds reported in these two papers, there were significant differences in the reported chemical shifts for the alkene protons. In contrast, the ^{13}C NMR data reported for **7** and **18** agreed apart from the expected differences in chemical shift in the vicinity of C-6 and C-7. The NMR data for compound **10** matched closely that of **7** apart from minor chemical shift differences caused by the change in substituent at C-7. The optical rotation of **10** was +60.8, compared to the value of –49.9 for isocyanide **18** and +60.5 for isothiocyanate **7**. Since literature precedent shows that R–NC and the corresponding R–NCS compounds have closely similar optical rotation values,[4,13] the new isocyanide was proposed to have a (6*R*, 7*S*) configuration. Isolation of the isothiocyanate **7** from the same extract strongly supported this stereochemical conclusion. On treatment with elemental sulfur and with catalytic amounts of selenium and Et_3N in THF, isocyanide **10** was converted to an isothiocyanate product whose ^{1}H and ^{13}C NMR data were identical with those of **7**.

Cadinene **4** was first described by our group from a Heron Island collection of *A. cavernosa*.[4] The same compound was subsequently reported from a Fijian collection of *Phakellia carduus* by Wright,[23] but the NMR data of the two samples differ. Notably, the ^{13}C NMR signal for C-10 was reported at δ_C 64.7 for the Heron Island compound and at δ_C 61.1 for the Fijian compound. The corresponding isocyanide **19** has δ_C 60.7 for C-10.[24] In RNC/RNCS pairs, the chemical shift of an isothiocyanato-substituted carbon is usually between 3 and 4 ppm downfield of the value for the equivalent isocyano-substituted carbon.[7,11] On this basis, the Heron island data better match the suggested structure. Although axisonitrile-3 **11** and axisothiocyanate-3 **14** are frequently reported from *Acanthella* spp., they remain poorly characterized in the literature.[13] Consequently, full NMR assignments are reported for both compounds in the Experimental Section.

The chemistry of Tani's Reef samples was next compared with those collected at two other dive sites, Coral Gardens and Mudjimba Island. Samples of *A. cavernosa* collected at Coral Gardens provided isothiocyanates **8** and **20**[25] and isocyanides **11** and **12**. A second collection from this site provided isothiocyanates **3**, **8**, **17**, **20**, and **21**,[11] isocyanides **11**–**13**, and the aromadendrane isocyanate **22**. This last metabolite was first reported by Braekman et al. without any detailed spectroscopic characterization,[26] and the relative stereochemistry initially proposed, along with that for the corresponding isocyanide, deduced by chemical comparison with the terpene alcohol palustrol.[27,28] A subsequent stereochemical reconsideration for palustrol[28] led to the revised stereochemistry shown here for **22**. The ^{1}H and ^{13}C NMR data of the Braekman isocyanide[27] match those reported elsewhere[7] for isocyanide **12**. Samples of *A. cavernosa* collected at Mudjimba Island provided isocyanate **22**, together with isothiocyanates **3**, **8**, **17**, and **20** and the cadinene isothiocyanate **23**.[29] The isocyanides isolated were **11**–**13**.

From a chemical perspective, the most valuable aspects of this study are that all the collections analyzed contained aromadendrane isothiocyanates **3** and **8**, the spiroaxane isocyanide **11**, and aromadendrane isocyanide **12**, while four out of the five collections contained the spiroaxane isothiocyanate **14** (Table 3). Although the amounts of the major compounds isolated from each collection differed, we consider the presence of these four compounds to be taxonomically representative of *A. cavernosa* at Mooloolaba. The five specimens collected at Mudjimba Island were all chemically identical, whereas sponges collected at Tani's Reef or Coral Gardens showed greater chemical diversity.



Intraspecies variation in sponge chemistry represents a significant challenge to the reisolation of pharmacologically useful lead compounds for development studies. The reasons for chemical

diversity within a given sponge species are likely complex and can be related to genetic[30] and environmental factors (including light and depth),[31,32] a response to predation[33] or infection,[34] competition for nutrients,[35] or the involvement of microbial symbionts.[30,32,36] Transplant experiments have shown that chemical variation in diterpene composition in the tropical marine sponge *Rhopaloeides odorabile* may be related to environmental factors such as light rather than to genetic differences per se.[31] However a study on the diterpene chemotype of *A. cavernosa* that was kept in aquaria for extended periods showed little chemical difference from wild sponges.[37] Speculation on a role for microorganisms in the biosynthesis of terpenes in *Acanthella* sponges is premature for two reasons. First, the symbiont populations of these sponges have not been adequately described, and second, the ability to synthesize terpenes has not been ascribed to marine microorganisms. In a number of sponge genera, terpenes have been shown to be localized within sponge cells rather than within symbionts.[36,38,39] It is clear from our study that, while there are major metabolites consistently produced by *Acanthella cavernosa*, there are as yet unknown factors that trigger the production of other metabolites in some specimens and not in others. Whether these represent genetic variants of the sponge or a response to subtle triggers such as co-located organisms, predation, trace elements or nutrient content of the water, water temperature, or sunlight exposure is a matter for further investigation. Finally, seasonal variation is a factor that could not be addressed in the current study, but which has been documented to impact on sponge chemistry or toxicity.[40–42]

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a Perkin-Elmer 241-MC polarimeter. One- and two-dimensional NMR spectra were acquired using Bruker AMX-400, Bruker DRX-500, or Bruker DMX-750 instruments. NMR spectra were obtained in deuterochloroform or deuterated methanol at room temperature. Samples were internally referenced to $CHCl_3$ at δ_H 7.25 and δ_C 77.0 or MeOH at δ_H 3.30. High- and low-resolution electron impact mass spectrometry (EIMS) were recorded on a Kratos MS25RFA mass spectrometer with an ionizing voltage of 70 eV. Gas chromatography/mass spectrometry (GC/MS) were recorded on a Hewlett-Packard 5890A gas chromatograph, carrying a DB5 capillary column in tandem with a Hewlett-Packard 5970 mass selective detector or a Shimadzu GCMS-QP5050A gas chromatograph mass spectrometer, carrying a Zebron ZB-5 capillary column (30 cm × 0.32 mm i.d.; × 0.25 μm df; 5% phenyl polysiloxane) with a Shimadzu AOC-20i auto injector. Retention times were obtained using the following temperature ramping program: initial oven temperature 50 or 100 °C; isothermal time 3.0 min, ramp 16 °C min^{-1}; final oven temperature 270 °C.

Animal Material. Specimens of *Acanthella cavernosa* Dendy 1922 (order Halichondrida, family Dictyonellidae) were collected at a depth of 10–20 m from Tani's Reef or Coral Gardens dive sites, both part of the Gneerings reef, Mooloolaba, or from Mudjimba Island, offshore from Maroochydore (Australia). The Tani's Reef and Coral Gardens sites are approximately 3 km offshore and less than 1 km apart from each other, whereas Mudjimba Island is 1 km offshore and 5 km distant from the Gneerings Reef sites. The samples were transported to the University of Queensland on ice, where they were stored at −20 °C until analysis. Voucher samples held at The Queensland Museum are for specimens collected at Tani's Reef (QM G322184) and Coral Gardens Dive Site (QM G319567). Although already well-described in the literature, a brief taxonomic description of the Queensland populations is appropriate here. Growth form is subspherical to flabellate, digitate or globular, with a honeycombed, reticulate construction. Color varies from bright orange, pale orange, or light brown alive. Oscules are large, located between surface conules, surrounded by membranous "lip" flush with surface. Texture is rubbery. Surface ornamentation consists of prominent conules, sharpish, interconnected by ridges, and cavernous. Ectosomal skeleton is membranous, collagenous, but with tips of styles protruding singly, regularly spaced. Choanosomal skeleton consists of axially compressed fibers, with degree of compression varying among specimens cored by strongyles (light fibers nearly fully cored) with extra-axial styles embedded in the axial skeleton and ascending to the surface. Collagen in the mesohyl varies from light to moderately heavy. Megascleres consist of axial strongyles, sinuous or curved (287−609 × 2−11.5 μm), and extra axial styles, straight or curved (271−453 × 3−12 μm). Variability within populations of this species mainly concern the density of the axial skeletal compression (looser in specimen G322184; more compressed in G319567); development of collagen in the mesohyl (lighter in G322184 and heavier in G319567); and thickness or degree of silicification of megascleres (thinner in G322184 and thicker in G319567). Nevertheless, this variation is not considered to be significant or to differentiate either as not conspecific.

Extraction and Isolation of Metabolites: Tani's Reef. Ten frozen sponges (8.3 g each) were chopped finely and extracted with CH_2Cl_2/MeOH, 1:1 (3 × 50 mL each). The extracts were filtered through a cotton wool plug, evaporated to aqueous suspension, and partitioned between H_2O (20 mL) and ethyl acetate (3 × 70 mL). The organic layers were combined, dried with anhydrous $MgSO_4$, and evaporated to give dark orange crude extracts. On the basis of GC/MS analysis and ^1H NMR data, six crude extracts were combined into a single sample named TR1 (501 mg), and four crude extracts were combined into a single sample named TR2 (281 mg). Each sample was then subjected to SiO_2 flash chromatography using a solvent gradient (100% hexanes to 5:1, 1:1, 1:5 hexanes/CH_2Cl_2 to 100% CH_2Cl_2 to 5:1, 1:1, 1:5 CH_2Cl_2/EtOAc to 100% EtOAc to 100% MeOH).

TR1 Sample. The 100% hexanes fraction contained sesquiterpene hydrocarbons including 9-aristolene (9.9 mg). The combined fractions eluting with 100% hexanes and hexanes/CH_2Cl_2 (5:1) were further purified by NP HPLC (two columns in series, 0.25% EtOAc/hexanes, 2 mL/min) to give the new 10-isothiocyanatoguai-6-ene (**1**) (0.2 mg) and a mixture of axisothiocyanate-2 (**2**) and 1-isothiocyanatoaromadendrane (**3**) (9.7 mg). The next fractions eluting with hexanes/CH_2Cl_2 (5:1) were further purified by NP HPLC (two columns in series, 0.25% EtOAc/hexanes, 2 mL/min) to give a mixture of the two 10-isothiocyanato-4-cadinenes (**4** and **5**) (6.9 mg), a mixture of acanthene B (**6**) and 7-isothiocyanato-7,8-dihydro-α-bisabolene (**7**) (4.6 mg), 10α-isothiocyanatoalloaromadendrane (**8**) (1.3 mg), and 10-isothiocyanato-4-amorphene (**9**) (3.8 mg). NP HPLC (two columns in series, 0.5% EtOAc/hexanes, 2 mL/min) of a portion of the hexanes/CH_2Cl_2 (1:1) flash column fraction gave axisonitrile-3 (**11**) (0.6 mg), 1-isocyanoaromadendrane (**12**) (0.9 mg), 7-isocyano-7,8-dihydro-α-bisabolene (**10**) (3.0 mg), and 11-isocyano-7*βH*-eudesm-5-ene (**13**) (0.8 mg).

10-Isothiocyanatoguai-6-ene (1): colorless oil; $[\alpha]_D$ +18.4 (*c* 0.043, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2110 (–NCS) cm^{-1}; ^1H and ^{13}C NMR see Table 1; GC/MS *m/z* $[M]^+$ 263 (28), 248 (7), 205 (9), 161 (86), 105 (100); HREIMS *m/z* 263.1706 $[M]^+$ (calcd for $C_{16}H_{25}NS$, 263.1708).

7-Isocyano-7,8-dihydro-α-bisabolene (10): yellow oil; $[\alpha]_D$ +60.8 (*c* 0.049, $CHCl_3$); IR ($CHCl_3$) ν_{max} 2136 (–NC) cm^{-1}; ^1H and ^{13}C NMR in $CDCl_3$ see Table 2; ^1H NMR (C_6D_6) δ 5.26 (1H, dd, 1.5, 4.0, H-4), 5.04 (1H, br m, H-10), 2.13 (1H, m, H-9a), 2.02 (1H, m, H-9b), 1.96 (1H, m, H-5eq), 1.80 (1H, m, H-5ax), 1.75 (2 × 1H, m, H-2), 1.65 (3H, s, H-12), 1.57 (3H, s, H-14), 1.53 (3H, s, H-13), 1.52 (1H, m, H-1eq), 1.46 (1H, m, H-8a), 1.40 (1H, m, H-6), 1.27 (1H, m, H-8b), 1.12 (1H, m, H-1ax), 0.94 (3H, t, 2.0, H-15); ^{13}C NMR (C_6D_6) δ 133.4 (C-3), 132.2 (C-11), 123.6 (C-10), 120.4 (C-4), 63.1 (C-7), 42.1 (C-6), 38.6 (C-8), 30.8 (C-2), 26.5 (C-5), 26.0 (C-12), 23.7 (C-1), 23.30 (C-15), 23.27 (C-14). 22.9 (C-9), 17.5 (C-13); GC/MS *m/z* $[M]^+$ 231 (2), 216 (7), 205 (2), 204 (12), 121 (56), 93 (100); HREIMS *m/z* 231.1976 $[M]^+$ (calcd for $C_{16}H_{25}N$, 231.1987).

Preparation of Synthetic 7-Isothiocyanato-7,8-dihydro-α-bisabolene (7). A sample of 7-isocyano-7,8-dihydro-α-bisabolene **10** (1.3 mg, 5.6 μmol) in dry THF 0.5 M (0.011 mL) was added into a mixture of elemental S (0.2 mg, 6.7 μmol), a catalytic amount of Se (5 mol %), and freshly distilled triethylamine (1.36 mg, 0.0134 mmol).[43] The mixture was refluxed for 2 h at room temperature, then the deposited Se was removed by filtration. Removal of triethylamine and THF *in vacuo* afforded the desired 7-isothiocyanato-7,8-dihydro-α-bisabolene (**7**) (1.4 mg, 93%) as an amber oil: $[\alpha]_D$ +23.7 (*c* 0.015, $CHCl_3$), lit.[10] +60.5 (*c* 6.8, $CHCl_3$); ^1H and ^{13}C NMR identical to data for the natural compound.[10]

Axisonitrile-3 (11): [13] pale white solid; mp 99–102 °C, lit.[13] 101–103 °C; $[\alpha]_D$ +43.4 (*c* 0.006, $CHCl_3$), lit.[13] $[\alpha]_D$ +68.4 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.11 (1H, br s, H-4), 3.57 (1H, br s, H-6), 2.21 (2H, m, H-2a, H-2b), 1.93 (2H, m, H-1a, H-1b), 1.77 (2H, m, H-8a, H-10), 1.72 (3H, br s, H-14), 1.57 (1H, m, H-11), 1.48 (1H, m, H-9a), 1.33 (1H,ddd, 4.0, 13.0, 13.0, H-8b), 1.13 (1H, m, H-7). 1.04

(1H, m, H-9b), 0.92 (3H, d, 6.6, H-12), 0.89 (3H, d, 6.6, H-13), 0.74 (3H, d, 7.0, H-15); ^{13}C NMR ($CDCl_3$) δ 155.6 (br, C-16), 144.8 (C-3), 123.6 (C-4), 64.5 (C-6), 57.0 (C-5), 43.8 (C-7), 35.8 (C-2), 34.9 (C-1), 34.3 (C-10), 31.2 (C-9), 29.7 (C-11), 24.9 (C-8), 20.7 (C-12), 20.3 (C-13), 16.9 (C-14), 16.0 (C-15); GC/MS *m/z* 231 [M^+], 216, 205.

Axisothiocyanate-3 (14):.[3,6,13] pale yellow oil; $[\alpha]_D$ +152.3 (*c* 0.003, $CHCl_3$), lit.[13] $[\alpha]_D$ +165.2 (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 5.11 (1H, q, 2.1, H-4), 3.67 (1H, br s, H-6), 2.22 (2H, m, H-2a, H-2b), 1.90 (2H, m, H-1a, H-1b), 1.79 (1H, m, H-8a), 1.72 (3H, br s, H-14), 1.68 (1H, m, H-10), 1.52 (2H, m, H-9a, H-11), 1.24 (2H, m, H-7, H-8b), 1.08 (1H, m, H-9b), 0.91 (3H, d, 8.3, H-12), 0.89 (3H, d, 8.3, H-13), 0.75 (3H, d, 8.3, H-15); ^{13}C NMR ($CDCl_3$) δ 144.8 (C-3), 129.2 (C-16), 123.9 (C-4), 67.3 (C-6), 58.9 (C-5), 45.3 (C-7), 35.9 (C-2), 35.0 (C-1, C-10), 31.3 (C-9), 30.1 (C-11), 25.4 (C-8), 20.8 (C-12), 20.4 (C-13), 16.9 (C-14), 16.1 (C-15); GC/MS *m/z* 263 [M^+], 248, 205.

TR2 Sample. The 100% hexanes fraction contained sesquiterpene hydrocarbons including 9-aristolene (4.3 mg). The combined fractions eluting with 100% hexanes and hexanes/CH_2Cl_2 (5:1) (17.4 mg) were further purified by NP HPLC (two columns in series, 0.25% EtOAc/hexanes, 2 mL/min) to give a mixture of axisothiocyanate-3 (**14**) and axisocyanate-3 (**15**) (1.8 mg), the new 10-isothiocyanatoguai-6-ene (**1**) (1.1 mg), 1-isothiocyanatoaromadendrane (**3**) (6.6 mg), a mixture of 10-isothiocyanato-4-cadinenes (**4** and **5**), and 10α-isothiocyanatoalloaromadendrane (**8**) (0.4 mg) The next fraction eluting with hexanes/CH_2Cl_2 (5:1) contained acanthene B (**6**) (1.1 mg). The fraction eluting with hexanes/CH_2Cl_2 (1:1) was further purified by NP HPLC (two columns in series, 0.5% EtOAc/hexanes, 2 mL/min) to give axisonitrile-3 (**11**) (4.2 mg) and 1-isocyanoaromadendrane (**12**) (1.0 mg).

Extraction and Isolation of Metabolites: Coral Gardens. Frozen sponge (22.3 g) was chopped finely and extracted as before to give a dark orange crude extract (225 mg). When chromatographed on SiO_2, the 100% hexanes fraction contained sesquiterpene hydrocarbons including (−)-9-aristolene (46.2 mg). The combined fractions eluting with 100% hexanes and hexanes/CH_2Cl_2 (5:1) contained a mixture of 10α-isothiocyanatoalloaromadendrane (**8**) and (1*R*,5*R*,6*R*,8*S*)-dec[4.4.0]ane-1,5-dimethyl-8-(1′-methylethyl)-5-isothiocyanate (**20**) (1.8 mg). The hexanes/CH_2Cl_2 (5:1) flash column fraction contained a mixture of axisonitrile-3 (**11**) and 1-isocyanoaromadendrane (**12**) (49.0 mg). Extraction of a second sample (225 g) collected at the same site yielded isothiocyanates **3** (1.8 mg), **8** (1.9 mg), **14** (10 mg), **20** (0.2 mg), and **21** (1.1 mg), isocyanides **11** (121 mg), **12** (124 mg), and **13** (40 mg), and the aromadendrane isocyanate **22** (0.7 mg).

1-Isocyanatoaromadendrane (22):[26] colorless oil; ^{1}H NMR ($CDCl_3$) δ 2.19 (1H, m, H-4), 1.91 (2 × 1H, m, H-3a, H-8a), 1.88 (1H, m, H-2a), 1.67 (1H, t, 11.0, H-5), 1.46 (1H, m, H-3b), 1.37 (3 × 1H, overlapping m, H-2b, H-9a, H-9b), 1.35 (1H, m, H-10), 1.05 (3H, s, H-13), 1.02 (1H, m, H-8b), 0.99 (3H, s, H-12), 0.98 (3H, d, J = 7.3, H-14), 0.95 (3H, d, J = 6.6, H-15), 0.69 (1H, ddd, 6.5, 9.2, 11.0, H-7), 0.62 (1H, dd, J = 11.0, 9.2, H-6); sample decomposed during acquisition of ^{13}C NMR data; GC/MS *m/z* [$M]^+$ 247, 232, 219, 204, 176, 161, 122,107, 81, 67, 41; HREIMS *m/z* 247.1936 [$M]^+$ (calcd for $C_{16}H_{25}NO$, 247.1940).

Extraction and Isolation of Metabolites: Mudjimba Island. Five sponges (8.3 g each) were chopped finely and extracted as before to give dark orange crude extracts of between 100 and 150 mg in size. On the basis of the GC/MS analysis and ^{1}H NMR spectra, the five crude extracts were combined into a single sample, which was then subjected to SiO_2 flash chromatography using the previously described solvent gradient. The fraction eluting with 100% hexanes and hexanes/CH_2Cl_2 (5:1) contained sesquiterpene hydrocarbons including 9-aristolene (4.1 mg). The combined fractions eluting with hexanes/CH_2Cl_2 (5:1) and hexanes/CH_2Cl_2 (1:1) (14.3 mg) were further purified by NP HPLC using 0.25% EtOAc/hexanes with two HPLC columns in series (flow rate 2 mL/min) to give a mixture of axisothiocyanate-3 (**14**) and axisocyanate-3 (**15**) (1.1 mg), a mixture of 1-isothiocyanatoaromadendrane (**3**) and the cadinene **23** (3.6 mg), 10α-isothiocyanatoalloaromadendrane (**8**) (1.3 mg), and (1*R*,5*R*,6*R*,8*S*)-dec[4.4.0]ane-1,5-dimethyl-8-(1′-methylethyl)-5-isothiocyanate (**20**) (0.9 mg). The hexanes/CH_2Cl_2 (1:1, 1:5) fraction contained a mixture of axisonitrile-3 (**11**) and 1-isocyanoaromadendrane (**12**) (80.3 mg). The hexanes/CH_2Cl_2 (1:5) fraction contained 11-isocyano-7β*H*-eudesm-5-ene (**13**) (7.0 mg).

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Supporting Information Available: Figures S1–S6. ^{1}H and ^{13}C NMR spectra for compounds **1**, **10**, and **22** and photograph of *A. cavernosa* . This material is available free of charge via the Internet at http://pubs.acs.org.

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1–48, and previous articles in this review series.
(2) Blunt, J. W.; Copp, B. R.; Munro, M. H. G.; Northcote, P. T.; Prinsep, M. R. *Nat. Prod. Rep.* **2006**, *23*, 26–78, and previous articles in this review series.
(3) Dumdei, E. J.; Flowers, A. E.; Garson, M. J.; Moore, C. J. *Comp. Biochem. Physiol.* **1997**, *118A*, 1385–1392.
(4) Clark, R. J.; Stapleton, B. L.; Garson, M. J. *Tetrahedron* **2000**, *56*, 3071–3076.
(5) Fattorusso, E.; Magno, S.; Mayol, L.; Santacroce, C.; Sica, D. *Tetrahedron* **1975**, *31*, 269–270.
(6) Hirota, H.; Tomono, Y.; Fusetani, N. *Tetrahedron* **1996**, *52*, 2359–2368.
(7) Mayol, L.; Piccialli, V.; Sica, D. *Tetrahedron* **1987**, *43*, 5381–5388.
(8) Mitome, H.; Shirato, N.; Miyaoka, H.; Yamada, Y.; van Soest, R. W. M. *J. Nat. Prod.* **2004**, *67*, 833–837.
(9) Burgoyne, D. L.; Dumdei, E. J.; Andersen, R. J. *Tetrahedron* **1993**, *49*, 4503–4510.
(10) Sullivan, B. W.; Faulkner, D. J. *J. Org. Chem.* **1986**, *51*, 5134–5136.
(11) Ciminiello, P.; Fattorusso, E; Magno, S.; Mayol, L.; Pedone, C.; Santacroce, C.; Sica, D. *Can. J. Chem.* **1987**, *65*, 518–522.
(12) Burreson, B. J.; Christopherson, C.; Scheuer, P. J. *Tetrahedron* **1975**, *31*, 2–15–2018.
(13) Di Blasio, B.; Fattorusso, E.; Magno, S.; Mayol, L. *Tetrahedron* **1976**, *32*, 473–478.
(14) Stapleton, B. L. Ph.D. thesis, The University of Queensland, 2002, p 179.
(15) Jumaryatno, P.; Rands-Trevor, K.; Blanchfield, J. T.; Garson, M. J. *Arkivoc* **2007**, 157–166.
(16) Tada, H.; Tozyo, T.; Shiro, M. *J. Org. Chem.* **1988**, *53*, 3366–3368.
(17) He, H.; Faulkner, D. J.; Shumsky, J. S.; Hong, K.; Clardy, J. *J. Org. Chem.* **1989**, *54*, 2511–2514.
(18) Manzo, E.; Ciavatta, M. L.; Gavagnin, M.; Mollo, E.; Guo, Y.-W.; Cimino, G. *J. Nat. Prod.* **2004**, *67*, 1701–1704.
(19) Gulavita, N. K.; de Silva, E. D.; Hagadone, M. R.; Karuso, P.; Scheuer, P. J.; Van Duyne, G. D.; Clardy, J. *J. Org. Chem.* **1986**, *51*, 5136–5139.
(20) Kassühlke, K. E.; Potts, B. C. M.; Faulkner, D. J. *J. Org. Chem.* **1991**, *56*, 3747–3750.
(21) Fusetani, N.; Wolstenholme, H. J.; Matsunaga, S. *Tetrahedron Lett.* **1991**, *32*, 7291–7294.
(22) Fusetani, N.; Wolstenholme, H. J.; Shinoda, K.; Asai, N.; Matsunaga, S. *Tetrahedron Lett.* **1992**, *33*, 6823–6826.
(23) Wright, A. D. *Comp. Biochem. Physiol.* **2003**, *134A*, 307–313.
(24) Okino, T.; Yoshimura, E.; Hirota, H.; Fusetani, N. *Tetrahedron* **1996**, *52*, 9447–9454.
(25) König, G. M.; Wright, A. D.; Sticher, O.; Fronczek, F. R. *J. Nat Prod.* **1992**, *55*, 633–638.
(26) Braekman, J. C.; Daloze, D.; Moussiaux, C.; Stoller, C.; Deneubourg, F. *Pure Appl. Chem.* **1989**, *61*, 509–512.
(27) Braekman, J. C.; Daloze, D.; Deneuborg, F.; Huysecom, J.; Vandevyver, G. *Bull. Soc. Chim. Belg.* **1987**, *96*, 539–543.
(28) Braekman, J. C.; Daloze, D.; Stoller, C.; Declercq, J. P. *Bull. Chim. Soc. Belg.* **1989**, *98*, 869–875.
(29) Capon, R. J.; MacLeod, J. K. *Aust. J. Chem.* **1988**, *41*, 979–983.
(30) Ridley, C. P.; Bergquist, P. R.; Harper, M. K.; Faulkner, D. J.; Hooper, J. N. A.; Haygood, M. G. *Chem. Biol.* **2005**, *12*, 397–406.
(31) Thompson, J. E.; Murphy, P. T.; Bergquist, P. R.; Evans, E. A. *Biochem. Syst. Ecol.* **1987**, *15*, 595–606.
(32) Becerro, M. A.; Paul, V. J. *Mar. Ecol.: Prog. Ser.* **2004**, *280*, 115–128.
(33) Cronin, G.; Hay, M. E. *Ecology* **1996**, *77*, 2287–2301.
(34) Slattery, M. *Chemoecology* **1999**, *9*, 97–104.
(35) Fleury, B. G.; Coll, J. C.; Tentori, E.; Duquesne, S.; Figueiredo, L. *Mar. Biol.* **2000**, *136*, 63–68.
(36) Flatt, P. M.; Gautschi, J. T.; Thacker, R. W.; Musafija-Girt, M.; Crews, P.; Gerwick, W. H. *Mar. Biol.* **2005**, *147*, 761–774.
(37) Rodríguez, J.; Nieto, R. M.; Hunter, L. M.; Diaz, M. C.; Crews, P.; Lobkovsky, E.; Clardy, J. *Tetrahedron* **1994**, *50*, 11079–11090.

(38) Faulkner, D. J.; Harper, M. K.; Haygood, M. G.; Salomon, C. E.; Schmidt, E. W. In *Drugs from the Sea*; Fusetani, N., Ed.; Karger: Basel, 2000; pp 107–119.

(39) Garson, M. J.; Thompson, J. E.; Larsen, R. M.; Battershill, C. N.; Murphy, P. T.; Bergquist, P. R. *Lipids* **1992**, *27*, 378–388.

(40) Turon, X.; Becerro, M. A.; Uriz, M. J. *Oikos* **1996**, *75*, 33–40.

(41) Page, M.; West, L.; Northcote, P.; Battershill, P.; Kelly, M. *J. Chem. Ecol.* **2005**, *31*, 1161–1174.

(42) Abdo, D. A.; Motti, C. A.; Battershill, C. N.; Harvey, E. S. *J. Chem. Ecol.* **2007**, *33*, 1635–1645.

(43) Fujiwara, S.; Shin-ike, T. *Tetrahedron Lett.* **1991**, *32*, 3503–3506.

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